HYPERVITAMINOSIS D RICKETS: THE ACTION OF VITAMIN D.

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THE relationship between vitamin D and rickets is not as simple as might be supposed from the innumerable examples of the vitamin's beneficial effect in the treatment of rickets. This statement is amply verified by numerous observations on the ability of excessive vitamin D administration to cause a partial decalcification of the skeleton. The following experiment goes somewhat further, since it shows that the prolonged and excessive feeding of vitamin D to young rats not only prevents calcification from progressing in a normal fashion, but also results in the development in the long bones of a histological picture identical with one type of rickets. It furthermore shows that the action of the vitamin, either in causing a decalcification or in preventing normal calcification, is not performed through the agency of osteoclasts, a finding which becomes of considerable importance when certain widely-held theories of calcium metabolism are considered in its light.

MATERIAL AND METHODS.

Fourteen rats, 28 days old, were used in the experiment. Seven were employed as controls and fed only our stock laboratory diet. The remaining 7 received in addition to the stock diet, 1 or 2 drops of activated ergosterol, 10,000 X (about 25,000 D) per day throughout the experiment. If an animal was gaining weight, 2 drops were given, if the animal was losing or remaining constant in weight only 1 drop was given. Two of the animals receiving ergosterol died on the 7th day, 1 on the 12th day, 1 on the 21st day, and the remaining 3 were killed on the 22nd day of the experiment. Although the control animals gained weight throughout (from approx. 45 g. to 110 g.), the animals receiving the ergosterol remained at a fairly constant weight of approximately 43 g.

The femora and humeri of both the controls and experimental animals were removed, fixed in formalin, decalcified in 5 p.c. nitric acid, embedded in paraffin and sectioned longitudinally. About 150 sections were stained with hæmatoxylin and eosin or Giemsa's stain and examined.

OBSERVATIONS ON THE LONG BONES.

It was obvious from a gross examination that the bones of the experimental animals were much shorter than those of the controls. Sections of the bones of the control animals revealed no evidence of any abnormality in the areas in which the normal longitudinal growth of bone occurs. Sections of the bones of the animal which died on the 21st day of the experiment and those

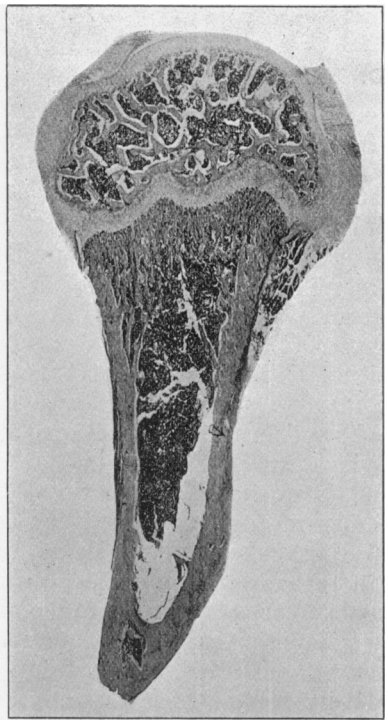

FIG. 1.

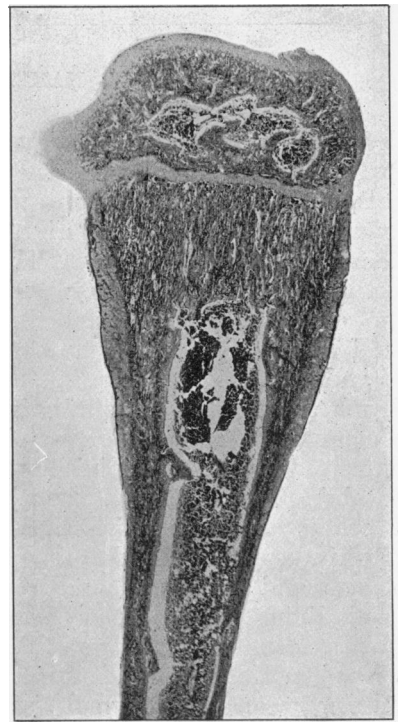

FIG. 2.

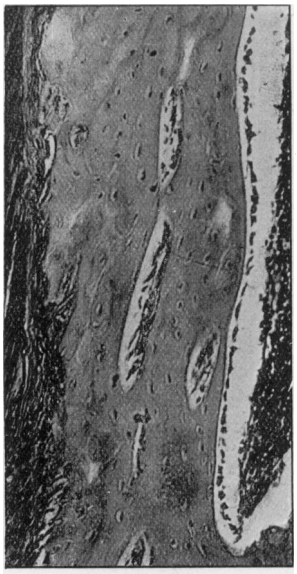

FIG. 3.

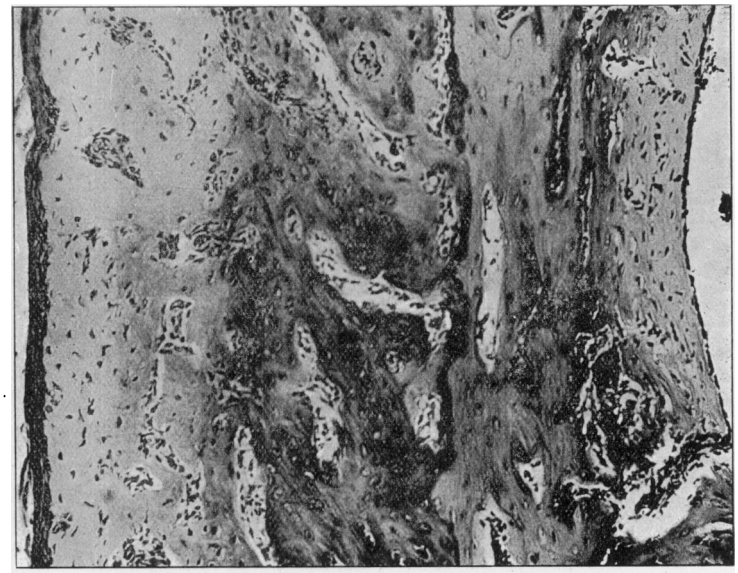

FIG. 4.

FIG. 1.—Photomicrograph (× 8) of end of humerus of control rat.

FIG. 2.—Photomicrograph (× 8) of end of humerus of rat which received enormous daily doses of activated ergosterol. The thin epiphyseal plate, the increased number of trabeculæ, the flattening of the epiphysis and the presence of large quantities of osteoid tissue are points of interest in this illustration.

FIG. 3.—Photomicrograph (× 240) of the cortex of the shaft of the humerus seen in Fig. 1. (Control.)

FIG. 4.—Photomicrograph (× 240) of the cortex of the shaft of the humerus seen in Fig. 2 (experimental). The periosteum appears at the left side of the figure and under it may be seen a large amount of osteoid tissue. Osteoid tissue may also be seen under the endosteum, which appears at the right side of the photomicrograph. The original cortex of the shaft may be seen running in a longitudinal direction through the picture near its right side.

which were killed on the 22nd day of the experiment presented a histological picture in the growing zone of the long bones, which was uniform in all the animals, and which it is thought, merits a detailed description.

As will be readily seen in Fig. 2, the epiphyses were somewhat flattened in their longitudinal diameter. Their framework was composed of cancellous bone, but both the number and arrangement of the trabeculæ as well as the character of their matrix varied from the normal. In the zone situated about the periphery of the epiphyses, the trabeculæ were very thick and numerous, while in the zone situated near the centre of the epiphyses there were a few relatively large marrow spaces. The trabeculæ were composed of a type of matrix which showed gradual transitions from fairly normal bone to a bluish-staining cartilage-like tissue, and also to a pale-staining osteoid tissue. Some of the cells were normal-appearing bone-cells, others associated with the bluish-staining matrix resembled cartilage cells, while the cells of the osteoid tissue resembled to some degree both fibroblasts and bone-cells, and were found in rather large flattened lacunæ. The cells lining the trabeculæ were for the most part of an osteogenic type. The larger marrow spaces were filled with hæmopoietic tissue and the smaller ones between the thick and numerous trabeculæ were filled with a primitive vascular type of mesenchymal tissue. Relatively few osteoclasts were seen.

The epiphyseal discs of the experimental animals were not as thick as those of the controls (Figs. 1 and 2) and the columns of cartilage cells in them were slightly irregular.

The zone of trabeculæ situated between the epiphyseal plate and the marrow cavity proper of the diaphysis occupied a much longer portion of the diaphysis than the corresponding zone in the bones of the control animals (Figs. 1 and 2). Numerous large vascular spaces were noted directly on the diaphyseal side of the epiphyseal plate, but the great part of the tissue situated between the epiphyseal plate and the marrow cavity proper was composed of trabeculæ which were irregular as to size, shape and staining reaction. These trabeculæ demonstrated the characteristics already described for those of the epiphyses, in that they were composed of matrix of bony, cartilaginous and osteoid varieties, with many transition stages between all three types. The trabeculæ were lined by osteogenic cells and osteoblasts. Only an occasional osteoclast was in evidence.

The cortex of the shaft was recognized by means of its compact structure. It was covered both on its exterior and interior surfaces by dense plates of pale-staining osteoid tissue, which were situated under the periosteum and endosteum respectively (Fig. 4). Both the osteoid tissue and the original cortical bone were permeated by large spaces filled with osteogenic cells, osteoblasts and blood-vessels, and in some instances spaces in the cortical bone were filled with osteoid tissue.

INTERPRETATION OF OBSERVATIONS.

The presence of osteoid tissue.—Osteoid tissue is in one sense poorly calcified bone and its presence indicates that the normal mechanism which accounts for calcification is at fault. It has been shown, however (Shipley, Kramer, and

Howland, 1926; Robinson and Soames, 1930), that tissue from rachitic bones will calcify properly *in vitro* if the cells are alive and if the fluid in which the tissues are immersed contains sufficient available mineral salts. It may therefore be concluded that the reason osteoid tissue is not well calcified is because its environment is not suitable for calcification.

The overgrowth of matrix.—The relatively tremendous amounts of matrix in the growing zones of the bones can best be explained by the well-known phenomenon of compensatory hyperplasia. It would seem that one is justified in concluding that the excessive production of matrix was due to the defective calcification of the matrix which was being produced throughout the course of the experiment.

The resorption of the cortical bone.—The scanty amount of true cortical bone in the shafts of the experimental animals indicate that a resorption of bone formed previously had occurred during the experiment. This finding, together with the points already discussed, illustrates that conditions during the experiment were not only unsuitable for proper calcification of newly-forming matrix, but were such as to institute a loss of mineral from the matrix already calcified before the experiment was begun.

DISCUSSION.

The first point to consider is whether or not the lesions produced in this experiment should be classified as rickets. They fulfil the requirements postulated by Mallory (1923) inasmuch as they demonstrate abundant evidence of defective calcification and compensatory overgrowth. The lesions, while differing in some respects from the type produced by feeding rats a high-calcium low-phosphorus diet, are very similar to the type produced by Pappenheimer, McCann and Zucker (1922) by feeding rats a high-phosphorus, low-calcium diet. These workers classified the latter condition as an atypical form of rickets. Therefore, as the lesions due to hypervitaminosis D satisfy the histological requirements for the diagnosis, and are similar to lesions already placed in this category, it would seem that they can rightly be considered as illustrating a type of rickets.

The second point which merits discussion is the fact that vitamin D was found to inhibit calcification of matrix, but not through the agency of osteoclasts. This ability to prevent the normal calcification of matrix appears still more curious when it is remembered that hypervitaminosis D is associated with a hypercalcæmia. It has been already pointed out that rachitic bones avidly attract calcium, as shown by experiments *in vitro*, so that it can be reasonably concluded that the effect of vitamin D in enormous doses is to cause the blood to attract calcium with greater force than the bones.

The manner in which vitamin D can increase the attraction of the blood for calcium remains somewhat obscure. Taylor, Weld, Branion and Kay (1931) have shown that overdosage effects of vitamin D and parathyroid hormone are similar and that vitamin D does not exert as toxic an effect in completely parathyroidectomized dogs as in normal ones. They therefore think that vitamin D acts on the parathyroid glands. Their work has received criticism at the hands of Dale, Marble and Marks (1932) who believe that

vitamin D does not exert its effect either on the parathyroid gland or on its hormone. The latter base their belief on experiments which show that complete parathyroidectomy did not prevent or significantly hinder the fatal intoxication produced by large doses of calciferol. They state, "The similarity of the symptoms and of the post-mortem appearances, produced by a fatal excess of either (parathyroid hormone or calciferol), seems to be adequately explained by the fact that a sufficient excess of calcium in the blood and tissues, whatever the cause, even if it is produced by continuous infusion of calcium salts, has this characteristic result, as demonstrated by Collip (1926)." This statement cannot, we think, be accepted in the face of the facts. Collip (1926) certainly showed that tremendous amounts of calcium could be injected into dogs without producing the picture of parathyroid intoxication. He showed, however, that this picture could be simulated by the administration of alternate injections of calcium chloride and sodium acid phosphate. The point is of interest because it suggests that the most striking features of parathyroid intoxication depend not on the height of the calcæmia, but on the precipitation of the calcium salts. There is certain evidence to indicate that the toxic effects of parathyroid hormone or vitamin D become most pronounced after the serum calcuim level has reached a peak and has started to fall. McLeod and Taylor (1925), and Collip, Clark and Scott (1925) both recorded this phenomenon, and Ham and Portuondo (1933) have shown that the calcification seen in hypervitaminosis D occurs on the fall of the serum calcium level after a single massive dose of vitamin D. Schour and Ham (1924) showed that the calcification of dentine was inhibited during the rise in the serum calcium level after a dose of either parathyroid hormone or vitamin D, only to be enhanced as the serum calcium level started downwards. Dale, Marble and Marks reported in detail their results on only one completely parathyroidectomized dog, and it died before the serum calcium level would normally have reached its peak. Consequently there seems to be good reason to consider that the dog died from some other cause than hypervitaminosis D, unless it can be shown by microscopic preparations that the dog showed the characteristic autopsy picture of hypervitaminosis D. The results on the other dogs of Dale, Marble and Marks's experiment, which were subjected to a less severe operation, do not negate Taylor, Weld, Branion and Kay's findings, because of the distinct probability of a considerable amount of parathyroid tissue remaining after operation. The parathyroid glands in the dog have a widespread and bizarre distribution, scattered lobules of this tissue being found as far down as the bifurcation of the trachea, Klotz (1922).

It is thus evident that uncertainty exists as to whether or not vitamin D acts on the parathyroid glands. If vitamin D acts through the parathyroid mechanism, it becomes, however, very difficult to believe that parathyroid hormone exerts its physiological effect by stimulating osteoclastic activity in the bones, a theory supported by Selye (1932), Thomson and Collip (1932), and Thomson and Pugsley (1932), because vitamin D was found in our experiment to inhibit calcification without the agency of osteoclasts. As a matter of fact the theory which postulates parathyroid hormone to act through osteoclasts is found on a hypothesis which has been, for the most part uncritically, accepted for so many years that it has gained all the prestige of a fact.

This hypothesis postulates osteoclasts to be active agents of bone resorption. There is nothing but circumstantial evidence to offer support for this supposition, and the circumstantial evidence indicates with more reason that osteoclasts are the result of bone disintegration rather than its cause (Ham, 1932*a*, 1932*b*). Moreover, Schour and Ham (1934) have shown that large doses of parathyroid hormone administered to rats exerted an inhibitory effect on the calcification of dentine as long as the serum calcium level was rising after the injection of the hormone. As there were no osteoclasts in the layer of cells adjacent to the dentine, and as the growing dentine is affected even more profoundly than bone by the administration of hormone, and as there is no definite evidence that osteoclasts are potent factors in bone resorption, there seems to be good reason to question the merit of the hypothesis which proposes that parathyroid hormone exerts its physiological effect by stimulating osteoclastic activity in the bones.

SUMMARY AND CONCLUSIONS.

Young rats, receiving very large daily doses of vitamin D showed rachitic lesions in their long bones after three weeks. As the matrix which formed in the bones during the experiment was very poorly calcified it was concluded that the administration of large amounts of vitamin D inhibited the normal calcification process in bone. As osteoclasts did not form a prominent part in the histological picture, the poor calcification of bone could not be attributed to them.

The phenomena observed in this experiment can best be explained, it is thought, by the theory which considers vitamin D to act by increasing in some way the attraction of the blood for calcium. The results are compatible with, although they do not directly support, the theory that vitamin D acts through the intermediary of the parathyroid mechanism to control a fraction of the serum calcium.

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AN EXPERIMENTAL STUDY OF THE POSSIBLE INFLUENCE OF INJURY IN THE GENESIS OF TUMOURS OF THE GONADS.

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IN the histories of patients with tumours of the testis, recent injury of the organ is frequently recorded ; and it is a widely held belief that such injury plays a part in the genesis of the growths. Thus, according to Thorek (1924), " such tumours are frequently the result of trauma " ; and Illingworth and Dick (1932) state, " trauma appears to play a very important part ". Dew (1925) finds that " there is a definite history of trauma in anything up to 50 per cent. of these tumours " ; and, while he admits that proof is lacking, he thinks that an " essential connexion " is strongly suggested. On the other hand, Hertzler (1931) warns against uncritical acceptance of trauma as a causative factor ; and Thomson-Walker (1932) also points out that the injury which is so often blamed " may only be the means of calling the patient's attention to what is a symptomless disease ".

The question is clearly one which can and should be investigated experimentally ; but, as far as I know, no such investigation has been recorded for any species of mammals. Michalowsky (1926, 1928 and 1929) claims to have produced teratomas in the testes of cocks by means of injections of zinc chloride solution ; but, in view of the not infrequent occurrence of abdominal teratomas in cocks (Masher, 1932), it is open to doubt whether Michalowsky's injections were really responsible for the growths which he observed. This doubt is increased by the remarkably brief intervals between the treatment and the appearance of the tumours in several of his cocks ; *e. g.* in one case, only 6 weeks after the injections, he found a teratoma 10 cm. in diameter, weighing 82 g., and containing well-differentiated cartilage, bone and glands.

The problem is not only one of great practical importance, but also one of great theoretical interest. If it were found to be possible, by some form of traumatic or chemical stimulation, to evoke teratomatous growth in gonadal